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# ON THE CLAIM THAT SOME TYPHOID- PARATYPHOID STRAINS SURVIVE THE MILK PASTEURIZATION

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The statement of Schorer and Rosenau<sup>1</sup> that the thermal death points of the pathogenic organisms that may render milk dangerous have been determined with precision in many laboratories and that an actual exposure to 60 C. for 20 minutes would suffice to kill such nonspore-bearing organisms as *B. diphtheriae*, *B. tuberculosis* and *B. typhosus* voiced the general conclusion of all who had worked on this subject. Although the practical application of these facts to the pasteurization of large volumes of milk was another problem, exposure to this temperature, for the time given, had been uniformly considered as adequate, and this aspect of the subject was looked on as closed.

Recently, however, Twiss<sup>2</sup> has reopened the subject and used bacteria of the typhoid-paratyphoid group as test organisms. Her contention is that, in actuality, the temperatures of pasteurization are inadequate; that the method of using small samples of the heated milk for the determination of the death of the bacterium present does not exclude the possibility of the survival of a few organisms. She asserts that if the whole test sample (100 c c) be incubated it will not infrequently be found that a few bacilli have survived exposure to 60 and even to 65 C. for 30 minutes.

The practical importance of the question immediately presented itself, and we began testing a series of cultures from different sources. Twenty-seven typhoid cultures recently isolated from carriers, 7 paratyphoid A cultures, 12 paratyphoid B. cultures and 4 enteritidis cultures were subjected to experiments, using the ordinary temperature of pasteurization.

Milk was sterilized in the autoclave, cooled and tested for sterility. It was then distributed in measured quantities in sterile containers. The containers were placed in wire baskets on a perforated tray in a water bath, and the temperature of the bath and the milk

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<sup>1</sup> Jour. Med. Research, 1912, 26, p. 127.

<sup>2</sup> Jour. Infect. Dis., 1920, 26, p. 165.

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raised to 60 C. After this temperature was reached, suspensions of the cultures in salt solution were poured into the milk. Rubber stoppers were inserted, the bottles shaken vigorously and quickly returned to the water bath in which they were completely submerged. The temperature of 60 was held during the pasteurization period of 15 minutes. Rapid cooling was accomplished by plunging the samples into a bucket of cold water held under an open faucet. This method of pasteurization excluded the preliminary heating of the bacteria while the temperature of the milk was being raised. In every one of these tests we used a volume of 100 c c of sterilized milk infected with the cultural growth from two 24-hour agar slants suspended in salt solution.

TABLE 1  
SUMMARY OF PASTEURIZATION EXPERIMENTS WITH TYPHOID, PARATYPHOID AND ENTERITIDIS STRAINS

Cultures Tested		Volume in C c of Milk Used in Tests	Infected With Growth From	Temperature of Milk When Infected	Minutes to Reach 60 C.	Minutes That Tests Were Heated at 60 C.	Results of Tests
B. typhosus	27 strains	100	2 agar slants	60 C.	—	15	No growth
B. paratyphosus A	7 strains	100	2 agar slants	60 C.	—	15	No growth
B. paratyphosus B	12 strains	100	2 agar slants	60 C.	—	15	No growth
B. enteritidis	4 strains	100	2 agar slants	60 C.	—	15	No growth
B. paratyphosus B	No. J* 202	100	2 agar slants	60 C.	—	15	No growth
B. paratyphosus B	No. J* 245	100	2 agar slants	60 C.	—	15	No growth
B. paratyphosus B	No. J* 252	100	2 agar slants	60 C.	—	15	No growth
B. paratyphosus B	No. J 202	400	10 agar slants	60 C.	—	10	No growth
B. paratyphosus B	No. J 202	400	10 agar slants	60 C.	—	20	No growth
B. paratyphosus B	No. J 245	400	10 agar slants	60 C.	—	10	No growth
B. paratyphosus B	No. J 245	400	10 agar slants	60 C.	—	20	No growth
B. paratyphosus B	No. J 252	400	10 agar slants	60 C.	—	10	No growth
B. paratyphosus B	No. J 252	400	10 agar slants	60 C.	—	20	No growth
B. paratyphosus B	No. J 202	300	1 agar slant	25 C.	17	10	No growth
B. paratyphosus B	No. J 202	300	1 agar slant	25 C.	17	20	No growth
B. paratyphosus B	No. J 245	300	1 agar slant	25 C.	18	10	No growth
B. paratyphosus B	No. J 245	300	1 agar slant	25 C.	18	20	No growth
B. paratyphosus B	No. J 252	300	1 agar slant	25 C.	10	10	No growth
B. paratyphosus B	No. J 252	300	1 agar slant	25 C.	10	20	No growth

\* Received from Dr. Jordan.

The inoculated milk samples were then incubated for 48 hours at 37 C. to allow multiplication of any organisms which survived. After incubation, samples in duplicate, 0.5 c c and 0.1 c c, were plated in agar and 2 broth tubes were each inoculated with 0.1 c c. Russell's medium was also inoculated from the infected milk, as well as from the inoculated broth tubes.

None of the micro-organisms tested survived a pasteurization period thus limited closely to 15 minutes at 60 C.

These experiments confirmed our previous experiences, as well as those of others. Whether or not there might be more resistant members of the typhoid-paratyphoid group could not be excluded as a possibility, although this seemed improbable. To exclude the possibility that Twiss was working with unusually heat resistant strains, we obtained some of the strains which in her experiments seemed to survive the usually accepted pasteurization period.

The resistance of these strains was tested in several ways. One set of experiments with 100 c c of milk was carried out as described, and no growth was obtained. Then, larger volumes were inoculated very heavily by using for each bottle the growth from 10 agar slants. Whether the milk was inoculated before or after reaching 60 C., the results were the same. No growth was obtained after 20 minutes or even after 10 minutes' heating at 60 C.

Repetitions of these tests, not recorded here, have given the same negative results.

It would seem that the apparent heat resistance of the strains reported by Twiss was due to the test method employed, namely, the use of cotton plugged flasks submerged to twice the depth of the milk. The possibilities of discordant results from this open method have been pointed out by Smith<sup>3</sup> and others.

#### CONCLUSIONS

There is no evidence that bacilli of the typhoid-paratyphoid group, even in small numbers, will survive heating to 60 C. for 20 minutes. To insure actual heating of all the sample and to exclude other sources of error in laboratory pasteurization tests, the sample container should be completely submerged in the bath.

<sup>3</sup> Jour. Exper. Med., 1899, 4, p. 233.